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Survey and genetic diversity of phytoplasmas from the 16SrV-C and -D subgroups in Hungary

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Abstract

In order to evaluate the risk represented by the wild reservoir as a possible source of 'flavescence dorée' outbreaks in Hungary, diverse wild perennial plants growing in vineyard areas were tested for the presence of 16SrV-C and D subgroup phytoplasmas. 16SrV phytoplasmas were detected by nested PCR-RFLP on the 16SrDNA in alders (86% infected) and in clematis (71% infected). Further characterisation by sequencing of the *map* gene revealed in both plants strains having the same *map* gene sequence as 'flavescence dorée' strains.

Key words: alder yellows, grapevine yellows, clematis, molecular typing, plant reservoir, *Mollicutes*.

Introduction

'Flavescence dorée' (FD) is a quarantine disease of grapevine spreading in south European vineyards. Although not reported in Hungary, it is present in the bordering country of Serbia (Duduk *et al.,* 2004). Because *Scaphoideus titanus* Ball, the vector of the phytoplasma, has been described in the south and in the centre of Hungary (Der *et al.,* 2007), there is an important risk of introduction of the disease in the country by infectious insects.

Another source origin for outbreaks could be the transfer of phytoplasmas from wild plants to grapevine. Indeed, phytoplasmas of the 16SrV-C and -D subgroups, genetically close to FD phytoplasmas, have been detected in alder (AldY phytoplasma) (Arnaud *et al.*, 2007; Malembic-Maher *et al.*, 2011) and in clematis (Filippin *et al.*, 2009). It has been shown that they can be transmitted to grapevine by occasional feeding of insect vectors living on these wild plants surrounding vineyards (Maixner *et al.*, 2000; Filippin *et al.*, 2009). With the presence of *S. titanus* in the vineyards, the phytoplasma could be spread epidemically.

In order to evaluate the risk originating from wild reservoirs, wild perennial plants collected in different parts of Hungary were screened for the presence of phytoplasmas of the 16SrV-C and -D subgroups. Phytoplasmas detected were further characterized by sequencing of the *map* gene.

Materials and methods

Between 2007 and 2009, 62 plant samples were collected in the Hungarian counties of Gyor-Sopron, Veszprem, Zala, Somogy, Pest and Heves (figure 1). Samples collected were asymptomatic *Alnus glutinosa*, *Clematis vitalba.* and other wild perennial plants showing symptoms of yellowing, reddening and, in some cases, dwarfism and small leaves. After total DNA extraction, samples were tested by nested PCR-RFLP on the 16SrDNA with the primers P1/P7 (Schneider *et al.* 1995) and R16F2n/R2 (Gundersen and Lee, 1996) followed by *Tru1*I digestion of the amplification products. Restriction profiles were compared with those of reference strains from the 16SrV and 16SrXII group. Samples detected positive for phytoplasmas of the 16SrV group were further characterized by nested-PCR followed by sequencing of the *map* gene as described in Arnaud *et al.* (2007). Sequence analyses, multiple alignment and phylogenetic tree (method of parsimony) were performed including reference *map* gene sequences of the 16SrV group previously described in Arnaud *et al.* (2007) and in Malembic-Maher *et al.* (2011).

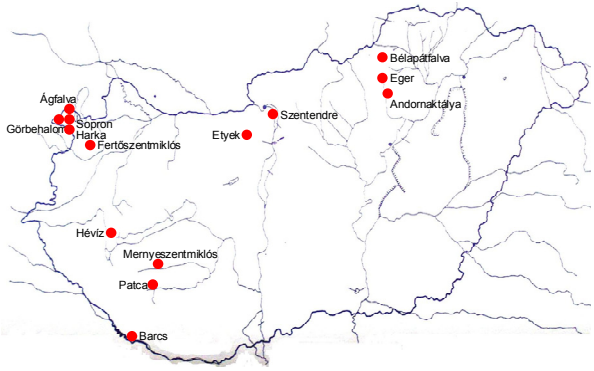

Figure 1. Localisation of the sampling sites in Hungary.

Results

Sequence typing and phylogenetic analysis based on *map* gene are presented in figure 2. Phytoplasmas of the 16SrV group were identified in 31 of the 36 alders tested. Among these, 22 samples were characterized on the *map* gene. Nine were found infected with a mix of AldY strains. Twelve were infected with

strains belonging to Map-AldY genetic clusters, some having the same *map* gene sequence as Palatinate grapevine yellows phytoplasmas from France and Germany (AM384890 and AM384892). One, B38, was infected by a strain belonging to the Map-FD1 cluster with a *map* genotype identical to FD and AlY strains from south-west France (AM238512). Ten of the 14 clematis were positive for 16SrV group phytoplasmas. The 6 strains genotyped all belonged to the Map-FD3 cluster and had the same *map* gene sequence as a FD strain from Italy (FN811141). None of the other 12 perennial wild plants were infected by 16SrV phytoplasmas, but one (*Fragaria sp.*) was infected by a 16SrXII group phytoplasma.

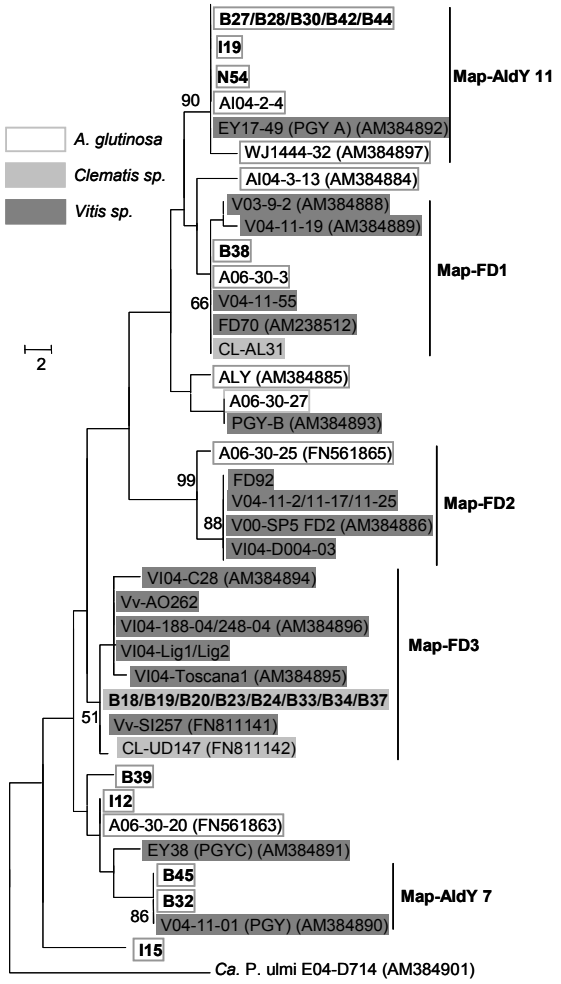

Figure 2. Phylogenetic tree constructed by *map* gene sequence analysis of the 16SrV group phytoplasma strains collected in Hungary. Hungarian strains are in bold and begin by the letters B, I and N. Reference strains are described in Arnaud *et al.* (2007) and Malembic-Maher *et al.* (2011).

Discussion

Genotyping studies have shown that phytoplasmas genetically closed to FD phytoplasma epidemic strains are present in Hungarian alders and clematis.

A recent survey conducted in 2010 has identified the presence of insect vectors *Oncopsis alni* and *Dictyophara europaea* which were shown to transmit these phytoplasmas from alders and clematis respectively, to grapevine (Maixner *et al.* 2000; Filippin *et al.* 2009). Characterisation of the strains present in the vectors is in progress. These findings show that the wild reservoirs such as alders and clematis constitute a risk for FD outbreaks in Hungary if *S. titanus* continues to colonize the vineyard.

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